Original Article

Unraveling the Temporal Pattern of Diet-Induced Insulin Resistance in Individual Organs and Cardiac Dysfunction in C57BL/6 Mice

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Type 2 diabetes is a heterogeneous disease characterized by insulin resistance and altered glucose and lipid metabolism in multiple organs. To understand the complex series of events that occur during the development of obesity-associated diabetes, we examined the temporal pattern of changes in insulin action and glucose metabolism in individual organs during chronic high-fat feeding in C57BL/6 mice. Insulin-stimulated cardiac glucose metabolism was significantly reduced after 1.5 weeks of high-fat feeding, and cardiac insulin resistance was associated with blunted Akt-mediated insulin signaling and GLUT4 levels. Insulin resistance in skeletal muscle, adipose tissue, and liver developed in parallel after 3 weeks of high-fat feeding. Diet-induced whole-body insulin resistance was associated with increased circulating levels of resistin and leptin but unaltered adiponectin levels. High-fat feeding caused insulin resistance in skeletal muscle that was associated with significantly elevated intramuscular fat content. In contrast, diet-induced hepatic insulin resistance developed before a marked increase in intrahepatic triglyceride levels. Cardiac function gradually declined over the course of high-fat feeding, and after 20 weeks of high-fat diet, cardiac dysfunction was associated with mild hyperglycemia, hyperleptinemia, and reduced circulating adiponectin levels. Our findings demonstrate that cardiac insulin resistance is an early adaptive event in response to obesity and develops before changes in whole-body glucose homeostasis. This suggests that obesity-associated defects in cardiac function may not be due to insulin resistance per se but may be attributable to chronic alteration in cardiac glucose and lipid metabolism and circulating adipokines.

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Type 2 diabetes has reached epidemic proportions, affecting >170 million people globally, and cardiovascular disease (CVD) is the leading cause of mortality in diabetes (1,2). The incidence of obesity, which increases the risk for development of type 2 diabetes and CVD, also continues to rise rapidly worldwide (3,4). The apparent triangular relationship of obesity, diabetes, and CVD may be interconnected by insulin resistance and altered glucose and lipid metabolism in response to insulin (5,6). Using genetic animal models of obesity, such as Zucker diabetic rats and leptin-deficient (ob/ob) mice, as well as diet-intervention models, many previous studies have reported that obese animals develop insulin resistance in skeletal muscle, adipose tissue, and liver (7–10). The mechanism underlying obesity-mediated insulin resistance involves the tissuespecific accumulation of fat and fatty acid metabolites and their deleterious effects on insulin signaling and glucose transport activity (11–13). An alternative mechanism is that adipocytes produce a host of metabolic hormones and inflammatory cytokines (adipokines), including resistin, adiponectin, leptin, tumor necrosis factor-α, and interleukin-6, and that the dysregulated production of adipokines alters whole-body insulin sensitivity (14–18). Thus, the underlying mechanism by which obesity causes insulin resistance remains unclear.

The heart is a constitutively energy-demanding organ, and normal cardiac function is dependent on a constant rate of ATP resynthesis by mitochondrial oxidative phosphorylation and, to a much lesser extent, glycolysis (19). Although mitochondrial lipid oxidation is the principal energy source, the maintenance of glucose utilization is necessary for normal cardiac function (20,21). This important role of cardiac glucose metabolism and insulin action was recently demonstrated in mice with cardiac-specific ablation of GLUT4 or the insulin receptor that developed cardiac hypertrophy and other phenotypes resembling the diabetic heart (22–24). Furthermore, studies using isolated perfused heart preparations, cultured cardiomyocytes, and positron emission tomography have uniformly shown insulin resistance in human and animal models of diabetic heart (25,26). In an important finding, cardiac insulin resistance was associated with diabetes independent of CVD, cardiovascular disease; GSK-3β, glycogen synthase kinase-3β; HGP, hepatic glucose production; 1H-MRS, 1H-magnetic resonance spectroscopy.

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TABLE 1
Metabolic parameters of C57BL/6 mice fed a high-fat diet for indicated time periods at basal state (fasted overnight) and during a 2-h hyperinsulinemic-euglycemic clamp

<table>
<thead>
<tr>
<th>Time</th>
<th>Body weight (g)</th>
<th>Plasma glucose (mmol/l)</th>
<th>Plasma insulin (pmol/l)</th>
<th>Basal HGP (µmol · kg⁻¹ · min⁻¹)</th>
<th>Plasma glucose (mmol/l)</th>
<th>Plasma insulin (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal period</td>
<td>Clamp period</td>
<td></td>
<td>Basal period</td>
<td>Clamp period</td>
</tr>
<tr>
<td>0 weeks</td>
<td>10</td>
<td>25 ± 1</td>
<td>8.5 ± 0.5</td>
<td>60 ± 15</td>
<td>131 ± 23</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td>1.5 weeks</td>
<td>8</td>
<td>28 ± 1*</td>
<td>8.4 ± 0.4</td>
<td>88 ± 16</td>
<td>103 ± 12</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>3 weeks</td>
<td>10</td>
<td>30 ± 1*</td>
<td>9.5 ± 0.7</td>
<td>104 ± 7*</td>
<td>95 ± 6</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>6 weeks</td>
<td>8</td>
<td>31 ± 1*</td>
<td>9.2 ± 0.3</td>
<td>122 ± 34*</td>
<td>100 ± 11</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>20 weeks</td>
<td>5</td>
<td>40 ± 2*</td>
<td>6.5 ± 0.5</td>
<td>72 ± 20</td>
<td>62 ± 4*</td>
<td>6.5 ± 0.5</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 vs. controls (high-fat diet for 0 weeks).

coronary artery disease, hypertension, and changes in coronary blood flow (27). A growing body of evidence further indicates that perturbations in cardiac energy metabolism and insulin resistance are among the earliest diabetes-induced alterations in the myocardium, preceding both functional and pathological changes (28–30). Despite the importance of insulin resistance in the diabetic heart, the mechanism by which cardiac insulin resistance develops is unknown.

To understand the complex series of events unfolding during the development of obesity-associated diabetes and CVD, the present study assessed the temporal pattern of insulin resistance in individual organs and cardiac dysfunction during chronic high-fat feeding in C57BL/6 mice. Using the in vivo techniques of hyperinsulinemic-euglycemic clamp 1H-magnetic resonance spectroscopy (1H-MRS), and echocardiography, we examined changes in insulin action, glucose/lipid metabolism, body composition, and cardiac function were determined in individual organs of awake mice.

RESEARCH DESIGN AND METHODS

Study 1: Changes in insulin action and glucose/lipid metabolism in individual organs during chronic high-fat feeding in C57BL/6 mice. Male C57BL/6 mice (n = 5–10) at age ~8 weeks were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under controlled temperature (23°C) and light (12 h of light [0700 –1900] and 12 h of dark [1900 – 0700]) conditions. Mice were fed a high-fat diet (55% fat by calories; Harlan Teklad, Madison, WI) ad libitum for 0 (control), 1.5 (10 days), 3, 6, or 20 weeks. The high-fat feeding was commenced at a different age for each group of mice in order to conduct experiments in age-matched mice (age 6–7 months). At least 4 days before the in vivo experiments, whole-body fat and lean mass were measured in awake age-matched mice using 1H-MRS (Bruker Mini-Spec Analyzer; Echo Medical Systems, Houston, TX). After their body composition was analyzed, the mice were anesthetized and surgery was performed to establish an indwelling catheter in the right internal jugular vein (31). On the day of the clamp experiment, mice were placed in a rat-sized restrainer, as previously described (31). All procedures were approved by the Yale University Animal Care and Use Committee.

Hyperinsulinemic-euglycemic clamps to assess insulin action in vivo. After mice were fasted overnight (~15 h), a 2-h hyperinsulinemic-euglycemic clamp was conducted with a continuous infusion of insulin (15 pmol · kg⁻¹ · min⁻¹), Humulin; Eli Lilly, Indianapolis, IN) and a variable infusion of 20% glucose (31). Basal and insulin-stimulated whole-body glucose turnover was estimated with a continuous infusion of [3-3H]glucose (PerkinElmer, Boston, MA) for 2 h before and throughout the clamps (31). To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-o-[1-14C]glucose (2-14C)DG, PerkinElmer) was administered as a bolus (10 µCi) 75 min after the start of the clamps. At the end of the clamps, the mice were anesthetized and their tissues were taken for biochemical and molecular analysis (31).

Biochemical and molecular assays. Glucose and insulin concentrations during clamps were measured as previously described (31). Plasma concentrations of [3-3H]glucose, 2-14C)DG, and H2O were determined after deproteinization of plasma samples. The radioactivity of H in tissue glycogen and tissue-specific 2-14C)DG-6-phosphate contents were determined as previously described (31). Heart samples were obtained at the end of clamps to assess insulin-mediated phosphorylation of Akt and total levels of GLUT4 (32). Insulin-stimulated rate of HGP during clamps was determined by subtracting the glucose infusion rate from whole-body glucose uptake. Whole-body glycolysis and glycogen plus lipid synthesis from glucose were calculated as previously described (31). Insulin-stimulated glucose uptake in individual tissues was assessed by determining the tissue (e.g., skeletal muscle, heart) content of 2-14C)DG-6-phosphate and the plasma 2-14C)DG profile. Insulin-stimulated glycolysis and glycogen synthesis in skeletal muscle were calculated as previously described (31).

Study 2: Changes in plasma adipokines and cardiac phenotypes in C57BL/6 mice fed a normal or high-fat diet. Longitudinal studies were performed in male, age-matched, C57BL/6 mice (obtained at age ~8 weeks) fed a normal (n = 5–10) or high-fat (n = 4–10) diet. A series of noninvasive experiments were performed in individual mice at 0, 1.5, 3, 10, 15, and 20 weeks of the respective diet feeding. Plasma fatty acids and triglyceride concentrations were determined using an acyl-CoA oxidase-based colorimetric kit (Wako, Richmond, VA) and triglyceride assay kit, respectively. Plasma levels of adiponectin, leptin, and resistin were measured using respective enzyme-linked immunosorbent assay kits (Linco, St. Charles, MO). M-mode echocardiography was performed using the Philips Sonos 5500 System with a 15-MHz probe in mice lightly anesthetized with inhaled isoflurane. The images were collected in the short and long axes; the data represent the averaged values of 3–5 cardiac cycles. Intramyocardial levels of fatty acyl-CoAs were measured using a PerkinElmer/Sciex API 3000 tandem mass spectrometer, as previously described (31).

Statistical analysis. Data are expressed as means ± SE. The significance of the difference in mean values between mice fed a high-fat diet for 0 weeks (controls) and those fed the diet for 1.5, 3, 6, or 20 weeks was evaluated using Duncan’s multiple range test (study 1). The significance of the difference in mean values between mice fed a high-fat or normal diet for 0 or 1.5 versus 3, 10, 15, or 20 weeks was also evaluated using Duncan’s multiple range test (study 2). The statistical significance was set at P < 0.05.

RESULTS

Basal metabolic parameters during chronic high-fat feeding. Body weight and whole-body fat mass, as measured by 1H-MRS in awake mice, were significantly elevated after 1.5 weeks (10 days) of high-fat feeding and further increased thereafter (Table 1 and Fig. 1A). In contrast, whole-body lean mass was not altered with high-fat feeding (Fig. 1B). Basal plasma glucose levels in overnight-fasted animals tended to increase after 3 and 6 weeks of high-fat feeding, but did not reach statistical significance (Table 1). Basal plasma insulin levels were increased by twofold after 3 and 6 weeks of high-fat feeding (Fig. 1C). However, after 20 weeks of high-fat feeding, plasma insulin fell to levels comparable with

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those of mice fed a normal diet, suggesting a decompensation of pancreatic β-cell functions (Table 1).

**Whole-body glucose metabolism during hyperinsulinemic-euglycemic clamps.** To determine the diet-induced changes in insulin action and glucose metabolism in vivo, a 2-h hyperinsulinemic-euglycemic clamp was conducted in awake, age-matched C57BL/6 mice fed a high-fat diet for various time periods. The steady-state rates of glucose infusion required to maintain euglycemia during clamps were significantly reduced after 3 weeks of high-fat feeding, at which time basal hyperinsulinemia also set in (Fig. 2A). Diet-induced whole-body insulin resistance was further demonstrated with [3H]glucose turnover during clamps, which showed a ∼30% decrease in insulin-stimulated whole-body glucose uptake after 3 weeks of high-fat feeding (Fig. 2B). Consistent with the changes in whole-body glucose uptake, insulin-stimulated whole-body glycolysis showed a tendency to be reduced after 3 and 20 weeks of high-fat feeding (Fig. 2D).

**Insulin-stimulated glucose metabolism in skeletal muscle, adipose tissue, and liver.** Organ-specific insulin action and glucose metabolism were assessed using 2[14C]DG, a nonmetabolizable glucose analog, during clamps. Insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius) was reduced by ∼30% after 6 weeks of high-fat feeding (Fig. 2C), and insulin-stimulated whole-body glycolysis showed a tendency to be reduced after 3 and 20 weeks of high-fat feeding (Fig. 2D).

FIG. 1. Basal metabolic parameters in C57BL/6 mice fed a high-fat diet for 0, 1.5 (10 days), 3, 6, or 20 weeks. A: Whole-body fat mass as assessed by 1H-MRS. B: Whole-body lean mass as assessed by 1H-MRS. C: Basal plasma insulin levels in overnight-fasted mice. Data are means ± SE for 5–10 experiments. *P < 0.05 vs. controls (high-fat diet for 0 weeks).

The basal HGP gradually decreased during high-fat feeding and was significantly reduced after 20 weeks of this diet (Table 1), a result that is consistent with the tendency for plasma glucose levels in overnight-fasted animals to decrease after 20 weeks of high-fat feeding. Insulin-mediated suppression of basal HGP (i.e., hepatic insulin action) was reduced by ∼40% after 3 weeks of high-fat feeding, and hepatic insulin resistance was further
exacerbated thereafter (Fig. 4C). By 20 weeks of high-fat feeding, insulin failed to suppress HGP completely; this effect was associated with a twofold increase in intrahepatic triglyceride levels (Fig. 4D) (34,35). In an interesting finding, the hepatic insulin resistance observed after 3 and 6 weeks of high-fat feeding was not associated with a significant alteration in hepatic triglyceride content, implying that other mechanisms are responsible for short-term, diet-induced hepatic insulin resistance.

Glucose metabolism and insulin signaling in the heart. Before the onset of whole-body insulin resistance, insulin-stimulated glucose uptake in the heart was significantly reduced (by ~30%) at 1.5 weeks of high-fat feeding and further decreased thereafter (Fig. 5A). By 20 weeks of high-fat feeding, insulin-stimulated cardiac glucose uptake was reduced by ~90% compared with controls not fed the high-fat diet. Diet-induced cardiac insulin resistance was associated with an ~40% decrease in insulin-stimulated Akt phosphorylation in the heart of mice fed a high-fat diet for 1.5 weeks (Fig. 5B), indicating that reduced cardiac glucose metabolism may be partly due to diet-induced defects in cardiac insulin signaling. Consistent with the previously shown role of Akt on peripheral glucose metabolism, these results indicate the important role of Akt in insulin-mediated cardiac glucose metabolism (36). Furthermore, myocardial levels of GLUT4 were determined in mice fed a high-fat diet; the results indicate that total GLUT4 expression levels were markedly reduced after 1.5 and 3 weeks of a high-fat diet (Fig. 5C). Taken together, these results indicate that diet-induced cardiac insulin resistance is due to defects in Akt-mediated insulin signaling and reduced expression of GLUT4. In another interesting finding, myocardial activity of AMPK was significantly elevated in mice fed a high-fat diet for 1.5 and 3 weeks, most likely due to increased leptin levels (Fig. 5D), but the total expression of AMPK was unaltered (data not shown).

Longitudinal changes in metabolic parameters with chronic high-fat or normal diet. Longitudinal studies were performed in additional groups of male, age-matched, C57BL/6 mice that were chronically fed a normal or high-fat diet. A series of noninvasive experiments were then performed in individual mice at 0, 1.5, 3, 10, 15, and 20 weeks of respective diet feeding. Whole-body fat mass was increased by twofold after 20 weeks of normal diet, reflecting age-associated change (Table 2). In contrast, whole-body fat mass was increased by twofold after 20 weeks of normal diet, reflecting age-associated change (Table 2). In contrast, whole-body fat mass was increased by twofold after 10 weeks of high-fat feeding and further increased to more than sixfold over basal (before high-fat feeding) levels after 20 weeks of fat feeding. On the other hand, whole-body lean mass showed a similar pattern of increase after a normal or high-fat diet (Table 2).

Circulating levels of glucose, fatty acids, and triglycerides were not altered during 20 weeks of normal diet. In contrast, the high-fat diet caused significant increases in plasma levels of fatty acids and glucose after 15 and 20 weeks of feeding, respectively (Fig. 6A and B). Diet-
induced onset of basal hyperglycemia, and thereby diabetes, after 20 weeks of high-fat feeding is consistent with reduced plasma insulin levels and is suggestive of blunted pancreatic β-cell response (i.e., decompensation of β-cells). Circulating triglyceride levels showed a tendency to be elevated after 15 and 20 weeks of high-fat feeding, but this difference did not reach statistical significance (Table 2).

Adiponectin, leptin, and resistin are adipocyte-derived circulating hormones that modulate insulin sensitivity, and leptin has been shown to play a role in the diabetic heart (15,16,37). Plasma adiponectin levels increased gradually over the 20 weeks of normal diet and were significantly elevated after 15 weeks of normal diet (Fig. 6C). These results are consistent with previously observed effects of aging on plasma adiponectin levels (38). High-fat feeding did not affect circulating levels of adiponectin; however, the age-associated increase in plasma adiponectin levels was blunted after 15 and 20 weeks of high-fat feeding (Fig. 6C). As a result, circulating adiponectin levels were significantly reduced after 20 weeks of high-fat feeding as compared with 20 weeks of a normal diet (Fig. 6C). In addition, when plasma adiponectin levels were normalized to whole-body fat mass, there was a significant reduction in the ratio of adiponectin to fat mass over the course of high-fat feeding as compared with normal-diet feeding (Fig. 6D). This finding suggests that although the absolute levels of plasma adiponectin did not differ with short-term feeding of either diet, high-fat feeding blunted the adipocyte production of adiponectin. Moreover, despite the gradual but significant increase in whole-body fat mass after 20 weeks of normal diet, plasma leptin levels were not altered in normal diet–fed mice (Table 2). In contrast, circulating leptin levels were markedly elevated after 1.5 and 3 weeks of high-fat feeding despite comparable whole-body adiposity to the normal diet–fed mice and remained elevated after 20 weeks of high-fat feeding (Table 2). In addition, circulating resistin levels were significantly elevated in the mice fed a high-fat diet for 1.5 and 3 weeks as compared with those fed a normal diet (Table 2).

**Longitudinal changes in cardiac function and structure with a chronic high-fat or normal diet.** To determine the effects of chronic high-fat feeding on cardiac function in vivo, the temporal pattern of changes in cardiac function was assessed using echocardiogram. Ventricular fractional shortening was not altered in C57BL/6 mice fed a normal diet for 20 weeks (Fig. 7A). In contrast, ventricular fractional shortening progressively declined after 10–15 weeks of high-fat feeding, with the difference reaching statistical significance after 20 weeks of fat feeding as compared with normal-diet feeding (Fig. 7A). In addition, ventricular fractional shortening was reduced in individual C57BL/6 mice fed a high-fat diet for 20 weeks as compared with the same mice after 3 weeks of a high-fat diet (Fig. 7B). Representative echocardiographs of mice fed a normal or high-fat diet for 20 weeks are shown in Fig. 7C and D.
The interventricular septal wall thickness at end-diastole was not altered after 20 weeks of normal or high-fat feeding as compared with after 3 weeks of the respective diet (Fig. 8A). In contrast, the left ventricular posterior wall thickness at end-diastole was significantly increased in mice after 20 weeks of high-fat feeding as compared with after 3 weeks of high-fat feeding, whereas it was not altered after 20 weeks of normal-diet feeding (Fig. 8B). Heart weight was significantly increased by $\sim20\%$ after 20 weeks of high-fat feeding as compared with after 20 weeks of normal diet feeding (Fig. 8C, left panel). However, when heart weight was normalized to whole-body lean mass, the results did not differ between the two groups of mice (Fig. 8C, right panel). Since myocardial accumulation of lipid metabolites has been associated with diabetic cardiomyopathy (39,40), we measured intramyocardial levels of fatty acyl-CoAs after 20 weeks of high-fat or normal diet. Intramyocardial levels of fatty acyl-CoAs were either significantly reduced ($C_{18:2}$ and $C_{18:3}$) or showed a tendency to be reduced after 20 weeks of high-fat feeding as compared with after 20 weeks of normal diet feeding (Fig. 8D). These results suggest that chronic high-fat feeding altered cardiac lipid metabolism and that increased levels of fatty acyl-CoAs were not responsible for altered cardiac functions. Furthermore, glycogen synthase kinase-3β (GSK-3β) is a ubiquitously expressed cardioprotective serine/threonine kinase, and altered expression or activity of GSK-3β has been shown to induce cardiac hypertrophy (41). Total protein levels of GSK-3β and phosphorylated GSK-3β were not altered in mice fed a high-fat diet for 1.5 or 20 weeks as compared with the respective mice fed a normal diet (data not shown), suggesting that GSK-3β–associated cardiac hypertrophic signaling may not be responsible for diet-induced alteration in cardiac function/structure.

**DISCUSSION**

Since the early observation of Sweeney (42) that dietary factors influence glucose tolerance, the causal relationship between obesity and insulin resistance has been exhaustively examined. What remains inconclusive is how lipid-mediated insulin resistance occurs in different organs and how it is related to cardiovascular complications. A study by Kraegen et al. (43) found that insulin resistance develops in liver and adipose tissue before it develops in skeletal muscle during chronic high-fat feeding in Wistar rats. In contrast, our results show that diet-induced insulin resistance in skeletal muscle, adipose tissue, and liver developed contemporaneously (after 3 weeks of high-fat feeding) in C57BL/6 mice. These discrepant findings may be due to the differences in animal species (rat versus mouse) and/or the high-fat diet used in each study. The significance of our findings is that they suggest that a common mechanism, secondary to increased adiposity, may be responsible for insulin resistance in individual organs in mice.

Obesity-associated insulin resistance may be due to...
intracellular accumulation of fatty acid-derived metabolites (i.e., fatty acyl-CoAs, diacylglycerol, and ceramide) that activate serine kinases (e.g., protein kinase C-β, IκB kinase-β) and downregulate insulin signaling and glucose transport activity (12,13,44). An alternative mechanism is that the altered adipocyte production of resistin and adiponectin seen in obese subjects may affect glucose metabolism and cause insulin resistance (15,16). In the present study, diet-induced insulin resistance in skeletal muscle was associated with a significant increase in intramuscular triglyceride content, which is consistent with the role of lipid and lipid metabolites on insulin signaling and in the glucose transport system (12,13). In addition, circulating levels of resistin were markedly elevated after 1.5 and 3 weeks of high-fat feeding and may further contribute to diet-induced insulin resistance in skeletal muscle (15). Our findings that plasma adiponectin levels were not altered with high-fat feeding indicate that adiponectin does not play an important role in the early stage of diet-induced insulin resistance in skeletal muscle. However, it should be noted that although the absolute level of plasma adiponectin was not altered with short-term high-fat feeding, the ratio of the adiponectin level normalized to the whole-body fat mass was significantly blunted with high-fat feeding. These results indicate that adipocyte production of adiponectin may be altered after

**TABLE 2**

Body composition and plasma triglyceride, leptin, and resistin levels in C57BL/6 mice fed a normal or high-fat diet for indicated time periods

<table>
<thead>
<tr>
<th>n</th>
<th>Fat mass (g)</th>
<th>Lean mass (g)</th>
<th>Triglyceride (mmol/l)</th>
<th>Leptin (ng/ml)</th>
<th>Resistin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>High fat</td>
<td>Normal</td>
<td>High fat</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4–10</td>
<td>5</td>
<td>4–10</td>
<td>5</td>
</tr>
<tr>
<td>0 weeks</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>18.3 ± 0.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1.5 weeks</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3 weeks</td>
<td>1.7 ± 0.2</td>
<td>3.1 ± 0.9</td>
<td>21.6 ± 0.8</td>
<td>0.24 ± 0.02</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>10 weeks</td>
<td>2.3 ± 0.2</td>
<td>3.7 ± 0.6</td>
<td>22.3 ± 0.5</td>
<td>0.28 ± 0.03</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>15 weeks</td>
<td>2.7 ± 0.3</td>
<td>5.4 ± 1.1</td>
<td>22.0 ± 0.4</td>
<td>0.28 ± 0.04</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>20 weeks</td>
<td>4.0 ± 0.4</td>
<td>11.4 ± 2.4</td>
<td>23.0 ± 0.5</td>
<td>0.25 ± 0.03</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 vs. mice fed a normal diet for comparable duration; †P < 0.05 vs. mice fed respective diet for 0 weeks. ND, not determined.
short-term fat feeding, but that the change is not significant enough to impact the circulating levels of adiponectin. Since insulin resistance developed in white adipose tissue after 3 weeks of high-fat feeding, altered insulin action and/or glucose metabolism in adipose tissue may be responsible for the blunted production of adiponectin.

In contrast to the findings in skeletal muscle, diet-induced insulin resistance in the liver developed before a significant increase in intrahepatic triglyceride content, a result that is consistent with our previous observation of unaltered fatty acyl-CoAs in the liver of mice fed a high-fat diet for 3 weeks (45). Contrary to our results, Samuel et al. (35) recently demonstrated that hepatic insulin resistance after 3 days of high-fat feeding was due to a profound increase in intrahepatic fat contents in Sprague-Dawley rats. The discrepant results may be due to the species difference: in rats, lipid partitioning in response to high-fat feeding may favor liver rather than skeletal muscle, with early insulin resistance developing in the liver before it develops in skeletal muscle, whereas in mice, lipids may be distributed more into skeletal muscle than the liver. To determine whether this phenomenon is associated with a higher level of physical activity in mice than in rats, more studies are clearly needed. It should also be noted that our results indicate that hepatic insulin resistance in response to short-term high-fat feeding may be due to other mechanisms in mice. In this regard, increased plasma resistin level may play a role in short-term, diet-induced hepatic insulin resistance. Consistent with this notion, Muse et al. (46) recently showed that 1-week treatment of specific antisense oligodeoxynucleotide directed against resistin mRNA normalized plasma resistin levels and reversed diet-induced hepatic insulin resistance.

A cohort of animal and human studies has indicated that the diabetic heart develops insulin resistance and that altered cardiac glucose metabolism plays an important role in the pathogenesis of diabetic heart failure (26–30). However, because most of these studies examined the heart of humans and animals with established diabetes, it is unclear what component of diabetes (e.g., hyperglycemia, hyperlipidemia) is responsible for cardiac insulin resistance. Thus, we assessed the changes in cardiac insulin action, glucose/lipid metabolism, and function during chronic high-fat feeding in C57BL/6 mice. Insulin-stimulated cardiac glucose metabolism was significantly reduced as early as 1.5 weeks after high-fat feeding began, before the onset of whole-body insulin resistance and hyperglycemia. Diet-induced cardiac insulin resistance was associated with blunted Akt activity and GLUT4 expression and may have been attributable to elevated myocardial triglyceride content, possibly mediated by activation of peroxisome proliferator-activated receptor-α (PPARα) (47,48). In this regard, mice with cardiac-specific overexpression of PPARα develop metabolic phenotypes similar to those of the diabetic heart, including increased lipid oxidation and insulin resistance (31,49). However, our finding that intramyocardial levels of fatty acyl-CoAs were not altered and, in fact, were reduced after high-fat feeding
feeding suggests that the lipotoxic effects of lipid metabolites are not the predominant mechanism of diet-induced cardiac insulin resistance. On the other hand, the increased plasma resistin levels seen with high-fat feeding may play a role in short-term, diet-induced cardiac insulin resistance. Thus, cardiac insulin resistance is an early adaptive event, secondary to increased lipid flux into cardiomyocytes and compensatory decrease in glucose metabolism, during the progression of obesity-associated diabetes; the underlying mechanism of this event needs to be further examined.

Our finding that cardiac insulin resistance is secondary to blunted Akt activity indicates that Akt, an insulin-signaling molecule downstream of the insulin receptor substrate and phosphatidylinositol 3-kinase, is an important mediator of cardiac glucose metabolism. In a paradoxical finding, mice with heart-specific overexpression of Akt were recently shown to develop ventricular hypertrophy associated with altered cardiac glucose metabolism (50). Thus, although reduced Akt activity is likely to play a role in diet-induced alterations in cardiac glucose metabolism, the chronic effects of Akt on cardiac function and structure remain unclear. In our study, insulin-stimulated cardiac glucose uptake decreased dramatically after 1.5–3 weeks of high-fat feeding (~60% change) despite comparable levels of Akt activity, suggesting that other factors may account for diet-induced cardiac insulin resistance. In this regard, our finding that total myocardial GLUT4 expression levels were markedly reduced after 1.5 and 3 weeks of high-fat feeding suggests the role of reduced GLUT4 and Akt-associated insulin signaling in diet-induced cardiac insulin resistance.

Although cardiac insulin resistance developed early in the course of diet-induced obesity, chronic and exacerbated defects in cardiac glucose metabolism (60–90% decrease after 6 and 20 weeks of high-fat feeding as compared with controls) may alter cardiac lipid metabolism, leading to a progressive decline in cardiac function. In this regard, diet-induced cardiac insulin resistance was associated with a dramatic increase in AMPK activity, possibly due to increased plasma leptin levels (33), which may upregulate lipid oxidation. The underlying mechanism may involve AMPK-mediated inhibition of acetyl CoA carboxylase and reduction of malonyl CoA, leading to an increase in mitochondrial activity of carnitine palmitoyl transferase 1 and lipid oxidation (51,52). In addition, cardiac insulin resistance and elevated lipid availability during high-fat feeding may directly increase the mitochondrial expression of uncoupling proteins 2 and 3, as was previously shown during lipid infusion in rat heart (53). Consistent with this notion, intramyocardial levels of fatty acyl-CoAs were significantly reduced in mice fed a
high-fat diet for 20 weeks despite elevated circulating fatty acid levels, suggesting increased intracellular lipid oxidation. With these data taken together, we hypothesize that increased AMPK activity and insulin resistance lead to chronically elevated lipid oxidation in the heart of high-fat-fed mice and that the subsequent mitochondrial or peroxisomal generation of reactive oxygen species may contribute to cardiomyopathy and cardiac dysfunction (40,54). Moreover, chronic, albeit mild, hyperglycemia and altered levels of adipokines may further contribute to diet-induced cardiac dysfunction.

Overall, our results demonstrate that insulin resistance develops in skeletal muscle, adipose tissue, and liver contemporaneously after 3 weeks of high-fat feeding in C57BL/6 mice and is associated with significant increases in intramuscular triglyceride and circulating resistin levels. Diet-induced cardiac insulin resistance develops before alterations in whole-body glucose homeostasis and secondary to defects in Akt-mediated insulin signaling and GLUT4 expression. Since cardiac function was not significantly blunted until after 20 weeks of high-fat feeding, our findings suggest that cardiac insulin resistance per se does not mediate cardiac dysfunction in high-fat-fed mice. Instead, chronically altered cardiac glucose metabolism and adipokines may exacerbate the deleterious effects of increased lipid oxidation, and lipotoxic-mediated cardiomyopathy may lead to a decline in cardiac function.

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